

In The Claims



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Please amend the claims as follows:

TECH CENTER 1600/2600

1. (Twice Amended) A method for discrimination and counting erythroblasts comprising the steps of:

(i) staining leukocytes in a hematologic sample by adding a fluorescent leukocyte binding antibody to the hematologic sample to bind the leukocytes;

(ii) raising the permeability only of cell membranes of erythroblasts in the hematologic sample to a nucleotide fluorescent dye which does not permeate a cell membrane when the permeability is not raised, the nucleotide fluorescent dye having a fluorescent spectrum that is distinguishable from that of a fluorescent labeling compound of the fluorescent labeled antibody in step (i);

(iii) staining nuclei of the erythroblasts in the hematologic sample with the nucleotide fluorescent dye;

(iv) analyzing the hematologic sample using flow cytometry to detect the nucleotide fluorescent signal of the stained erythroblasts and the fluorescent signal of the labeled antibody [signal of the leukocytes] bound to the leukocytes; and

(v) plotting the nucleotide fluorescent signal and the fluorescent labeled antibody signal in two coordinate axes to obtain a two-dimensional distribution chart discriminating between erythroblasts and leukocytes in the hematologic sample [and counting the erythroblasts from a difference in nucleotide fluorescent signal of the erythroblast and the fluorescent labeled antibody signal of leukocytes] based on the difference in the two-dimensional distribution chart

11 (ii)

and counting the erythroblasts [from a difference in nucleotide fluorescent signal of the erythroblast and the fluorescent labeled antibody signal of the leukocytes].

VI. claim 11

3. (Thrice Amended) The method according to claim 1 wherein [labeled leukocyte binding the fluorescent leukocyte of] the fluorescent labeled antibody in the step (i) comprises at least one compound selected from the group consisting of phycoerythrin, fluorescein isothiocyanate, allophycocyanin, Texas Red, [CY5 stands for a] arylsulfonate, cyanine fluorescent dye CY5, a peridinin chlorophyll complex, and a combination thereof.

4. (Twice Amended) The method according to claim 1, wherein the raising of the permeability of the cell membranes of erythroblasts in the hematologic sample to the nucleotide fluorescent dye in step (ii) comprises the steps of:

(i) admixing a first reagent fluid of hypotonic osmolarity containing a buffer for maintaining pH within an acidic range to the hematologic sample after the step (i); and

(ii) admixing thereto a second reagent fluid containing a buffer for neutralizing the first reagent fluid containing the hematologic sample and adjusting a mixture of the hematologic sample and the first reagent fluid to a pH wherein the leukocytes are stained] maintaining a pH from 5.0 to 11.0 and an osmolarity compensating agent for adjusting [the mixture to] an osmolarity [suitable] for retaining the shape and integrity of the leukocytes from 300 to 1000 mOsm/Kg H₂O.

10. (Twice Amended) The method according to claim 5, wherein the nucleotide fluorescent dye is used at a concentration within the range of 0.003mg/L to 10mg/L to form a mixture to be analyzed using flow cytometry to stain erythroblasts [according to